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Antimicrobial Activities of *Vernonia amygdalina* Against Oral Microbes

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Abstract: *Vernonia amygdalina* was assessed for active principles. The results showed that the extract of the plant possessed the active principles e.g. terpenoids, tannins, alkaloids, saponins and glycosides. The antimicrobial activity of the extracts was assayed against *Streptococcus mutans* and *Staphylococcus aureus*, using the agar diffusion method. The extract from *V. amygdalina* show a very high antimicrobial activity because they somehow have a very low pH value. The ethanolic extract of *V. amygdalina* has the lowest minimum inhibitory concentration on *Streptococcus mutans* at 25mg/ml while aqueous extract of *V. amygdalina* had the highest value of MIC on *Streptococcus mutans* at 55mg/ml. The ethanolic and aqueous extract of *V. amygdalina* had their MIC at 60mg/ml on *S. aureus* which was the highest minimum inhibitory concentration on *Staphylococcus aureus*. Ethanolic extract of *V. amygdalina* had the lowest value on *S. aureus* at 40mg/ml. On the whole, most of the sensitive extract were only able to inhibit the growth of the organism which is known as bacteriostatic in action while some exert a killing effect on the test organism and suggests that the extracts from plants were bactericidal.

Key words: *Vernonia amygdalina* · MIC · MBC · *Streptococcus mutans* · *S. aureus*

INTRODUCTION

Botany and medicine have been closely linked throughout history. Prior to this century, medical practitioners whether allopath [medical doctors], homeopaths, naturopaths, herbalist or shamans had to know the plants in the area and how to use them since many of their drugs were derived from plants [1]. Around 1900, 80% of the drugs were derived from the plants. However, in the decades that followed, the development of synthetic drugs from petroleum product caused a sharp decline in the pre-eminence of drugs from live plant sources [1]. But with the recent trend of high percentage resistance of microorganism to present day antibiotics [2], efforts have been intensified by researchers towards a search for more sources of antimicrobial agents.

Medicinal plants contain certain physiologically active principles, which over the years have been exploited in traditional medical practice for the treatment of various ailments [3]. Plants of both lower and higher groups are known to produce chemical substances with which they defend themselves against invading microorganisms. The chemical material or substances which are commonly referred to as antimicrobial agents are developed from the noticeable conditions such as viral disease attack, mammalian predation attack and the development of extra-ordinary array of defenses against chemicals and the struggles to survive under intense competition for resources and nutrients, [4]. The agents' chemical substance produced by plants and microorganism which elicit or exhibit inhibitory (bacteriostatic) effect or destructive (bactericidal) effect on other microorganisms [5].

Traditionally in Africa, stem and roots of some plants are used in cleaning teeth by chewing them into brush-like ends. Investments have revealed those chewing sticks contain some active principles which posses antimicrobial activities against oral microbial flora [6]. A number of African medicine plants were studies by Sofowora *et al*., 1982. Their studies showed that a petroleum extract of *Heteromorpha trifoliata* leaves had antifungal properties against cladosporium while the ethanolic extracts of

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Mormodica charantia, *Alstonia booner* and *Ocimum bacilium* were found to posses antimicrobial activity against *Escherichia coli*, *Salmonella paratyphi* and *Shigella dysenteriae.* A decoction of the root of the East African Plant *Ozoroa mucronata* is used for various folk medicinal treatments such as intestinal parasites, dysentery, diarrhea, gonorrhea, billharzia and abortion [7].

Aqueous extracts of roots of *Fagora zanthoxyloides* (pako atta) was reported to have significant anti-sickling effects on red blood cells of patients with sickle cell disease [8].

A quantitative assessment of the antimicrobial activity of garlic (*Allium sativum)*, was carried out by Rees *et al*. [9]. An aqueous extract of free dried garlic was incorporated into growth media which inhibited many representative bacteria, yeasts, fungi and a virus [12] demonstrated that citrus wastes are potential sources of biologically active principles. Their results with *Citrus reticulate* seed extracts show that the plants is active against some fungal which are *Fusarium soloni, Helminthesoorium satiuum, Alternaria soloni, Aspergillus Niger* and *Aspergillus flavus.*

Another plant of medicinal value used in the treatment of diseases is *Carica papaya* (Pawpaw) [11, 12]. Studied the antimicrobial substances from *Carica papaya* fruit extract. Ripe and unripe pawpaw fruit (epicarp, seeds and leaves) were extracted separately and purified. All the extracts that of the leaves produced very significant antibacterial activity on *Staphylococcus aureus, Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa and Shigella flexneri.* The active substance was bactericidal and showed properties of a protein.

More work carried out on *Allium sativum* (garlic) by Duke *et al*. [13] showed that it inhibited thirty-seven strains of Mycobacterium constituting of about seventeen species at various test concentrations; Akinyanju *et al*., [14] reported that hot water extract of the leaves of *Acalypha Torta* has significant inhibitory effect on *Staphylococcus aureus*, *Pseudomonas aeruginosa, Esherichia coli*, *Klebsiella* sp., *Acinetobater* sp. They also reported the effects of both ethanolic extracts of the leaves of *Acalypha torta,* the same range of organisms with the exception of *Pseudomonas aeruginosa* and *acinetobacter* sp Oloke *et al*., [15] reported that the essential oils of *Aframonium melegueta* fruits inhibited the mycelia growth of *Trichopyton mentagrophytes* at a pronounced rate but they inhibited the *Aspergillus niger* at lower rate. Saxena and Saxena [16] reported an active principle of the neem plant Azadirachtin with pharmaceutical properties confirming the medicinal values ascribed to the plant.

The antimicrobial properties of cumin (*Cumin cyminum*), a commonly grown Indian spice was studied by Shetty *et al*. [17]. This spices is a flovourant and consistent of household medicine. Unlike other spices, mould and yeast do not usually infest it. Moulds (*Penicillium* and *Asperillus*) and Yeast, *Saccharomyes* and *Candida* cultures were sensitive to cumin volatile oil and cuminaldhyde than bacteria. *Acalpha wilkesiana* has been screened and found to contain polyphenols from methanolic extracts, [18]. The polyphenols are shown to have greater antimicrobial activity against *Candida albicans*, *Esherichia coli* and *Staphylococcus aureus* than *sesquiterpenes*.

More interestingly, the polyphenol turned out to be more potent than orthodox antifungal drugs such as ‘canastein cream’ containing biphenyl-imido-zylimethane [19]. The successful utilization of *Acalypha wilkesiana* decotion to cure disease has stimulated research on its validity. The polyphenols are shown to have greater antimicrobial activity against *Candida albicans, Escherichia coli* and *Stayhylococcus aureus* than *sesquiterpenes*.

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Nwobu and Farva [20] determined the anti-candida activities of *Allium cepa* (onion) and *Allium sativum* (garlic) oils. In this analysis they extracted oils from the spices using petroleum ether and ethly remedies against disease. [21, 22] have reported the medicinal, insecticidal, antimicrobial, antiprotozal and antitumor properties of onion and garlic. Aqueous extracts of onions and garlic are known to possess an *in-vitro* growth inhibition effect against a large number of fungi including Yeasts [23] and to have protective effect against *in vivo* experimental fungal infections.

Plants that are tested for antibacterial property in this research is: *vernonia amygdalina; vernonia amygdalina* belongs to the family Compositae and was named after William Vernon a 17th century botanist [24]. It is particularly abundant in grasslands through the tropics ‘bitter taste. Its is a popular leafy vegetable among the Ibos of Eastern Nigeria.

The Yoruba name is "Ewuro jije", Olugbu' in Igbo and 'Shiwaka' in Hausa. The shrub is usually about 5m high. the leaves are simple and entire (5x15cm), finely glandular below and displaying few lateral nerves. The flowers occur in a panicle, white and fragrant. It is differentiated from its counterpart *V. colorata*, which grows wildly hairy leaves of the latter [25].

Vernonia amygdalina have been in many homes in the eastern and Western parts of Nigeria as food especially in the preparation of soups. The characteristic bitter taste is believed to have after taste of sweetness. The peeled stem is often used as chewing stick for cleaning the teeth and is very effective as anticaries [26]. The bitterness of the leaves is also exploited by nursing mothers to assist in weaning their babies by rubbing the juice on their breast [25]. It is also suggest to be useful to nursing mother as it improves lactation. The plants leaves and other parts have been used solely or mixed with other plants for the treatment of various suspected illnesses. Interaction with some traditional medicine healers in Ilorin metropolis revealed that the leaves have been extracted raw in aqueous solution and taken as anti-dysentery diction. It is also said to be useful for sugar level control in diabetes patients; A cup full daily is prescribed to patients in serious cases.

Despite the usefulness and closeness of *Vernonia amygdalina* to homes, [27], considered it as one of the unexploited crops of economic importance. The modern agriculturists regard it as weed on their farmlands. Little has been reported about the plant scientifically and almost no notice is given to its antimicrobial property, though its metabolites are vernodaline and vernomygsline [28]. Another of its chemical constituents is sesquiterpene lactone which is made up of vernodaline subunits. This accounts for the bitter taste and has been exploited to discover its antitumoral and antimicrobial activities [29]. *Staphylococcus aureus* and *Streptococcus mutans* are used in this study.

It is therefore the aim of this study is to verify the antimicrobial activity of *vernonia amygdalina* plant so as to find an alternative for the common antibiotics presently in use.

MATERIAL AND METHODS

Collection of Plant Materials: Fresh leaf isolates of *Vernonia amygdalina,* was purchased from oja oba market at Ilorin, Kwara State, Nigeria. The plant was identified at the Herbarium of the Department of Plant Biology University of Ilorin.

Collection and Maintenance of the Test Organisms: Two bacterial isolates were used for the tests. They were collected from the Department of Microbiology of University of Ilorin. The organisms are *Streptococcus mutans and Staphylococcus aureus*. All the bacterial species used were maintained on nutrients agar slopes and stored in the refrigerator at a temperature of 4°C from where they were subcultured unto fresh media at regular intervals.

Processing of the Plant: The plant was sundried. The plant was then pounded in a mortar and further ground to powder using electric blender of model W-BL5085M and stored in airtight containers.

Sterilization Methods: All glassware were properly washed and dried before being sterilized. They were sterilized in an hot air oven at 170°C for 2 hours. Each of the materials was wrapped with aluminium foil before sterilization. Distilled water was sterilized in the autoclave at 121°C for 15mins. Cork borer and glass rods were sterilized by dipping into 70% alcohol prior to flaming in bunsen burner. The work bench was swabbed with 75% alcohol before and after each experiment.

Preparation and Standardization of Bacterial Inoculum: Standardization of bacterial inoculum was done by picking five colonies of each organism into nutrient broth and incubated at 37°C for 18-24hrs.Turbidity produced was adjusted to match 0.5 McFarland standard (10^8cfu/ml) which was further adjusted 10^5 cfu/ml [30].

Preparation of Plant Material and Plant Extracts: Four different extractants namely cold ethanol, hot ethanol (28°C), cold water and hot water (28°C) were used for the plant.

Reconstitution and Sterilization of Extracts: The dried powdered leaf was measured into McCartney bottles and appropriate volume of the extractant was added to make a stock solution of 200mg/ml. The stock solution was then sterilized using 0.65 membrane filter by suction pump. The sterilized extract from *Vernonia amygdalina* was stored inside sterile McCartney bottle and kept in the refrigerator at 7°C until used for the antibacterial test. The extracts were tested for sterility by plating it on nutrient agar and incubated for 24hrs at 37°C.

Test for Antimicrobial Activity of the Extracts: Sterile nutrient agar plates were prepared and allowed to solidify. Standardized organism of 0.1ml of a day old were

introduced into the plates and sterile cotton swab was used to spread the inocula evenly on the surface of the agar and the excess drained off. The plates were left on the bench for 1hour so that the inocula will diffuse into agar. A sterile cork borer of 5mm diameters was used to make 5 ditches on the plates. Varying concentrations of the extracts, i.e. 200mg/ml, 100mg/ml, 25mg/ml were made and 0.5ml of the extract was dropped in each of the appropriately labeled plate and control were set up for each plate by adding 0.5ml of the appropriate solvent into the 5th ditch. The plates were duplicated and left on the bench for few minutes for the extract to diffuse into the agar and later incubated at 37°C for 24hours. After incubation the zone of clearance around each ditch was measured using a metric ruler by taking measurement from the edge of the plate to the point where the growth of the organism started. The diameter of the zone of inhibition which represents antibacterial activity was measured.

Determination of Minimum Inhibitory Concentration (MIC) of the Extract: The broth dilution method was used to determine MIC. Varying concentrations of the extracts were used which, ranged from 5mg/ml – 200mg/ml each concentration contain 0.1ml was added to each 9ml of nutrient broth containing 0.1ml of standardized test organism of bacterial cells. The tubes were incubated aerobically for 24hours at 37°C. Controls were equally set up by using solvent and test organisms without the extract.

Determination of Minimum Bacterial Concentration (MBC): A sample from the tubes used in MIC (Minimum inhibitory concentration) determination which did not show any visible growth after the period of incubation were streaked on nutrient agar (NA) plates. The lowest concentration of the extract indicating a bacterial effect after some hours of aerobic incubation at 37°C was regarded as the minimum bactericidal Concentration (MBC).

Phytochemical Screening of the Leave Extracts: Phytochemical screening was done in order to detect the presence of plant constituents such as alkaloids, tannins, saponins, phenolics, glycosides and plobatannis in the plant extract. Ethanolic extracts of the plant was prepared by macerating known weight of the plant in ethanol. The extract was then filtered using Whatmann No.1 filter paper. A portion of the extract was used to test for the following plant constituents: alkaloids, saponins, tannins, phlobatannins, flavonoids and glycosides using the methods described below:

Test for Saponins: Two milliliter of the aqueous and ethanolic extracts in a test tube was shaken for two minutes. Frothing which persisted on shaking was taken as evidence for the presence of saponins [31].

Test for Alkaloids: Three milliliter of ethanolic or aqueous extract was stirred with 5ml of 1% HCl on a steam bath for twenty minutes. The solution obtained was cooled and filtered and to the filtrate was added few drops of Mayer's reagent/Picric acid. A cream precipitate indicates the presences of alkaloid [31].

Test for Phenolics: Two drops of 5% ferric chloride were added to five milliliter of the ethanolic and aqueous extracts in a test tube. A greenish precipitate was taken as an indication of phenolics [31].

Test for Tannins: One ml of freshly prepared 10% potassium hydroxide was added to 1ml of the ethanolic extracts and aqueous extracts. The presence of a dirty white precipitate was taken as indication of tannins [31].

Test for Steroids: Five drops of concentrated tetraoxosulphate VI acid was added to 1ml of the extract. Red colouration indicated the presence of steroids [32].

Test for Phlobatannins: Hydrochloric acid 1%, was added to 1ml of the ethanolic and aqueous extracts. A red precipitate was taken as the presence of phlobatannins [32].

Test for Flavonoids: To a volume of three milliliter of the ethanolic and aqueous extracts, a volume of 1ml of 10% sodium hydroxide was added. A yellow colouration indicated the presence of flavonoids[31].

Test for Glycosides: To 1ml of the ethanolic and aqueous extract, 2ml of chloroform was added. Tetraoxosulphate VI acid was carefully added to form a lower layer. A reddish brown colour at the interface indicated the presence of a steroid ring [31].

Table 1: Antimicrobial activity of *V. amygdalina* against *S. mutans* and *S. aureus*

	Aqueous extract (mg/l)					Ethanolic extract (mg/l)				
Test organism	C	25	50	100	200	C	25	50	100	200
S. mutans	-	0.5	2.00	3.00	3.50	-	2.00	3.00	6.00	10.50
S. aureus	-	3.00	5.00	6.00	7.50	-	-	1.00	2.00	4.50

Key: - = NO zone of inhibition, C= Control of each extract, Diameter of inhibition is in mm.

Table 2: Minimum Inhibitory and bactericidal Concentrations of plant extract on *Streptococcus mutans* and *Staphylococcus aureus*

Test organism	Plant	Plant extract	MIC	MBC (mg/ml)
Streptococcus mutans	*V. amygdalina*	Ethanol	30	50
		water	55	-
Staphylococcus aureus	*V. amygdalina*	Ethanol	45	125
		Water	60	-

Key: MIC = Minimum Inhibitory Concentration, MBC = Minimum Bactericidal Concentration - = Not sensitive

Table 3: Physical characteristics of *V. amygdalina*

Plant Extract	Sample	pH	Colour of the Extract
V. amygdalina	Ethanolic extract	3.5	Light green

Table 4: Phytochemical analysis of the *Vernonia amygdalina*

Samples/Tables	Ethanol	Water
Saponins	+	+
Cardiac glycosides	+	+
Tannins	+	+
Alkaloids	+	+
Flavonoids	-	-
Phylobatannins	-	-
Steroids	-	-
Phenolics	-	-

Key: + = Present, - = Absent.

RESULTS

Table 1 shows the antimicrobial activity of *V. amygdalina* against *S. mutans* and *S. aureus*. Table 2 show the minimum inhibitory and bactericidal concentrations of plant extract on *Streptococcus mutans* and *Staphylococcus aureus*. The results indicated that the ethanolic extract rather than the aqueous extract of *Vernonia amygdalina* produced effective antimicrobial activities. The antimicrobial activities of the extracts were determined by measuring the zones of inhibitions on the agar plates.

The determination of the antimicrobial activity of ethanolic extract with aqueous extract of the leaves of *Vernonia amygdalina* showed that the extract has antibacterial properties. *Staphylococcus aureus* was the most sensitive organism to the aqueous extracts of V*ernonia amygdalina* with the zone of inhibition of 7.50mm and the least sensitive organism to the same extract was *S*treptococcus *mutans* with the zone of inhibition of 3.50mm at the same concentration (i.e 200mg/ml). The ethanolic extract of V. *amygdalina* has the highest zones of inhibition on *S. mutans* with a diameter of 10.50mm while *Staphylococcus aureus* is less sensitive.

The MIC values obtained on the test organisms varied from one organism to another. The ethanolic extract of *V. amygdalina* has the lowest minimum inhibitory concentration on *Streptococcus mutans* at 25mg/ml while aqueous extract of *V. amygdalina* had the highest value of MIC on *Streptococcus mutans* at 55mg/ml. The ethanolic and aqueous extract of *V. amygdalina* had their MIC at 60mg/ml on *S. aureus* which was the highest minimum inhibitory concentration on *Staphylococcus aureus*. Ethanolic extract of *V. amygdalina* had the lowest value on *S. aureus* at 40mg/ml. The MBC result shows that *V. amygdalina* extract had bactericidal effects on *S. mutans* and *S. aureus* at 50mg/ml and 125mg/ml respectively.

Phytochemical Analysis of the Ethanolic Extract: The result of the phytochemical analysis of the ethanolic extract of *Vernonia amygdalina* leaf is shown in Table 4. Plant constituents such as saponins, cardiac glycosides,

tannins and steroids were detected while components such as alkaloids, flavonoids and phlobatannis were not detected.

DISCUSSION

Several investigators on medicinal plants have indicated that organic solvents such as alcohols are extensively used for crude extraction before being re-extracted to obtained purified active compounds using some other organic solvents [31, 33]. Several investigators had reported that plants contain antibacterial or antimicrobial substances [1, 2, 34, 35]. Their results show that there was variation in the degree of antibacterial activities of the extracts.

Ethanolic extracts showed more activity against the bacterial isolates than the water extracts. This may be due to the higher volatility of the ethanol which tends to extracts more active compounds from the samples than water. This is in concord with the observation of Ibekwe *et al*., [2].

The plant extracts from the two plants had profound activities against both Gram-positive and Gram negative bacteria. There was however, more activity against the Gram negative organism than the Gram positive. Pelczar *et al*. [5] suggested that the difference in susceptibility of Gram positive and Gram-negative bacteria to various antimicrobial agents probably depends on structural differences in their cell walls. For example, amount of peptidoglycan, presence of receptors and lipids, nature of cross linking, activity of autolytic enzymes that determined the penetration, binding and activity of the antimicrobial agents.

The marked difference in the effects of the extracts on the organism therefore, is suggestive of the activity against cell wall components of the organism. The antimicrobial substance appears to exert antimicrobial activity by inhibiting the growth of and by killing the sensitive bacteria. This particular finding was also encountered by Emeruwa [11] in his study on the antimicrobial substance from *Carica papaya* fruit extract.

The ethanolic extract has the largest zone of inhibition in all. This is probably due to the higher concentrations where cold aqueous extract had a higher activity from *V. amygdalina* against *S. aureus*. This is in accordance with the work of Ijeh and Adedokun [35]. One of the factors that affect microbial susceptibility is the concentration of the activity component; the more the concentration the higher the activity of the chemical substance.

The minimum bactericidal concentrate (MBC) from the *V. amygdalina* for *P. aeruginosa* was found to be 50mg/ml while *E. coil* exhibit a bacteriostatic activity because there was growth on the plates. However only the ethanolic extract exhibited bactericidal activity. The minimum bactericidal concentration was not detected for Aloe vera. This may be due to the fact that when Aloe plants is opened it immediately begins to oxidize which leads to reduction in potency [36].

Secondary compounds, which include tannis, saponins, cardiac glycosides and alkaloids were reported by Kaufman *et al*. [37] to be present in higher plants. Results of the preliminary phytochemical screening revealed the presence of these compounds in the extacts of *Vernonia amygdalina*. The compounds were reported by Kaufman *et al*. [37] o be indicative of the potential medicinal value of the plants in which they appear.

Since the organisms were affected in one way or the other by exposure to different extracts, it is very possible that at much higher concentrations and observable time limit, there could be bacterial effect on the organisms. The susceptibility of the entire test organism especially *S. mutans* that has resistance against many antibiotics goes further to prove that the plant have potentials as alternatives source of antimicrobial agents in the growing bacterial resistance world. It should be noted however, that just as there is the potentially advantageous medicinal use of this plant and to some extents some antimicrobial property, studies have shown that the leaf extract has induced abortion in goats and mice. The extract also reduced the rate of isolated frog heart and in cat caused marked fall in blood pressure [25]. However this should not be discouraging since many antibiotics that are in used today have their side effects.

From the result presented, the different concentrations of the extract have shown varying degree of antimicrobial activities against the test organisms. Table 3 shows how the pH of the extract varies on pH scale. However, the leaf extract have pH range between 3.0 to 6.9 which lies between and acidic range on the pH scale while some lies between a neutral range on the pH scale. Although, microorganisms will often grow over wide ranges of pH and far from their optima, there are limits to their tolerance. Drastic variation in cytoplasmic pH can harm microorganisms by disrupting the plasma membrane or inhibiting the activity of enzyme and membrane transport proteins [39]. Therefore, the acidity of some extract might have an effect on the test organism The extract from *V. amygdalina* show a very high

antimicrobial activity because they somehow have a very low pH value. On the whole, most of the sensitive extract were only able to inhibit the growth of the organism which is known as bacteriostatic in action while some exert a killing effect on the test organism and suggests that the extracts from plants were bactericidal.

CONCLUSION

The result obtained in this research work indicates that the leaves of *Vernonia amygdalina* contain antimicrobial substances which is both bacteriostatic and bacteriocidal on several species of bacteria. However the bacteria varied widely in their degree of susceptibility to the plant extracts. Through such research as this, natural product of plant origin have been obtained for human use. For instance, *Aloe vera* product from Ghana are a source of export earning for that country. Several herbal products are known to be produced in india today including herbal toothpaste which are in Nigeria markets.

Dudu Osun (Africa black soap) produced mainly in the Western part of Nigeria is a natural product of which the chief ingredient is sodium hydroxide (NaOH) or caustic soda obtained from plants. Recently, Dermacur lotion, Dermacur medicated soap, Dermacur ointment and Lamsterploxide A and B produced from plants are discovered by Researchers in Dermatology division of National Veterinary Research Institute (NVRI), Vom, Plateau state, Nigeria and have been put on sale.

The extract from the leaves of *V. amygdalina* should be further subjected to in-vivo trial after isolation and characterization of the active components as well as elucidation of structure of the components. A double blind trial should be conducted among infected individuals using this plant extracts and conventional standard antibiotics of choice for each of those diseases with which the extract had activity with the aetiology. Effort should be made to purify active component of the plants extract so as to standardize it in recommendable dosage form. This is of importance as it will remove the fear of over dosage toxicity and other side effects.

Sooner than we expect, we may be able to develop a broad- spectrum drug from *V. amygdalina* which will be able to cure human of various ailments and also the *V. amygdalina* will be able to establish itself as a drug that will serve in treatment of dental caries.

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